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ECOLOGY, REPRODUCTIVE BIOLOGY AND POPULATION GENETICS OF *OPHIOGLOSSUM VULGATUM* (OPHIOGLASSACEAE) IN MASSACHUSETTS

ROBERT T. MCMASTER

ABSTRACT

The northern adder's-tongue fern, *Ophioglossum vulgatum* var. *pseudopodum* (Blake) Farw. (Ophioglossaceae), occurs in five known populations in Massachusetts and has been designated as threatened by the Massachusetts Natural Heritage Program. The autecology, population biology and population genetics of *O. vulgatum* var. *pseudopodum* were analyzed during the summer of 1992. The distribution of each population was mapped, associated vegetation sampled, and permanent plots established for long-term study. Isozyme electrophoresis was employed to assess variability within and between populations. The historical distribution of the species in Massachusetts was examined based upon herbarium specimens. Each of the known Massachusetts populations is in an early successional site with a unique history of disturbance; sites include pasture, a power line right-of-way, and abandoned beaver meadows. Four of the five populations are small, ranging from 1 to 109 sporophytes. All specimens subjected to isozyme analysis were monomorphic for all loci examined. The implications of the spatial and temporal distribution, population size, and low genetic variability of *Ophioglossum vulgatum* var. *pseudopodum* are discussed. Management considerations for the preservation of this and other early successional species are suggested.

Key Words: *Ophioglossum vulgatum*, Ophioglossaceae, fern reproductive biology, population genetics, early successional species, rare plant species, self-fertilization, Massachusetts

INTRODUCTION

Ophioglossum vulgatum var. *pseudopodum* (Blake) Farw., the northern adder's-tongue fern (Ophioglossaceae), has a broad distribution, occurring throughout its range in a variety of wet and mesic habitats. In Massachusetts, *O. vulgatum* var. *pseudopodum* is known from over one hundred historical sites and as late as 1978 was thought to occur in all counties of the state (Coddington and Field, 1978). By 1985, however, with only four extant stations known, it was placed on the Massachusetts list of threatened species, a status which it holds at present (Massachusetts List of Endangered, Threatened and Special Concern Species, 1994). The species was listed as rare and endangered in Rhode Island in 1978 (Church and Champlin, 1978) and officially designated as state endangered by the Rhode Island Natural Heritage Program in

1992 (Rhode Island Natural Heritage Program, 1992). *Ophioglossum vulgatum* was listed as threatened in Connecticut in 1991 (Connecticut Department of Environmental Protection, 1991). It has not been granted special status by the other New England states.

This study was undertaken to investigate the ecology, reproductive status and population genetics of *Ophioglossum vulgatum* var. *pseudopodum* and its present distribution in Massachusetts. Specific objectives included (a.) examination of all known populations of *O. vulgatum* in the state, mapping of their distribution and estimation of population sizes; (b.) identification of associated vascular plant species in each site; (c.) analysis of mating systems and assessment of genetic variability for each population through starch gel electrophoresis; (d.) reassessment of the status of *O. vulgatum* in Massachusetts and identification of possible reasons for its apparent success in some locations and its decline in others; and (e.) development of management strategies for preserving the existing populations.

BIOLOGY OF OPHIOGLOSSUM VULGATUM

Gross Morphology, Taxonomy and Distribution

The Ophioglossales have traditionally been considered plants of "ancient lineage and extreme conservatism practically unparalleled in the other fern groups" (Clausen, 1938). Their sporophytes are homosporous, perennial herbs with a single, erect stem bearing one or more small sterile leaves and a single fertile branch with sporangia. The gametophytes are subterranean and nonchlorophyllous (Tryon and Tryon, 1982).

Ophioglossum includes about 30 species grouped in four subgenera and distributed worldwide from the tropics to the Arctic (Tryon and Tryon, 1982). Sporophytes of all *Ophioglossum* species have a short, erect stem 1–12 cm long. The stem bears a single leaf with a mature sterile lamina 1.6–25 cm or more in length. The leaf is usually entire and narrowly elliptic, but in a few species it may be palmately lobed or dichotomously branched. Many species of *Ophioglossum* display reticulate or areolate venation. The fertile branch usually arises at or below the base of the sterile lamina, often in the form of a spike with two rows of large sporangia.

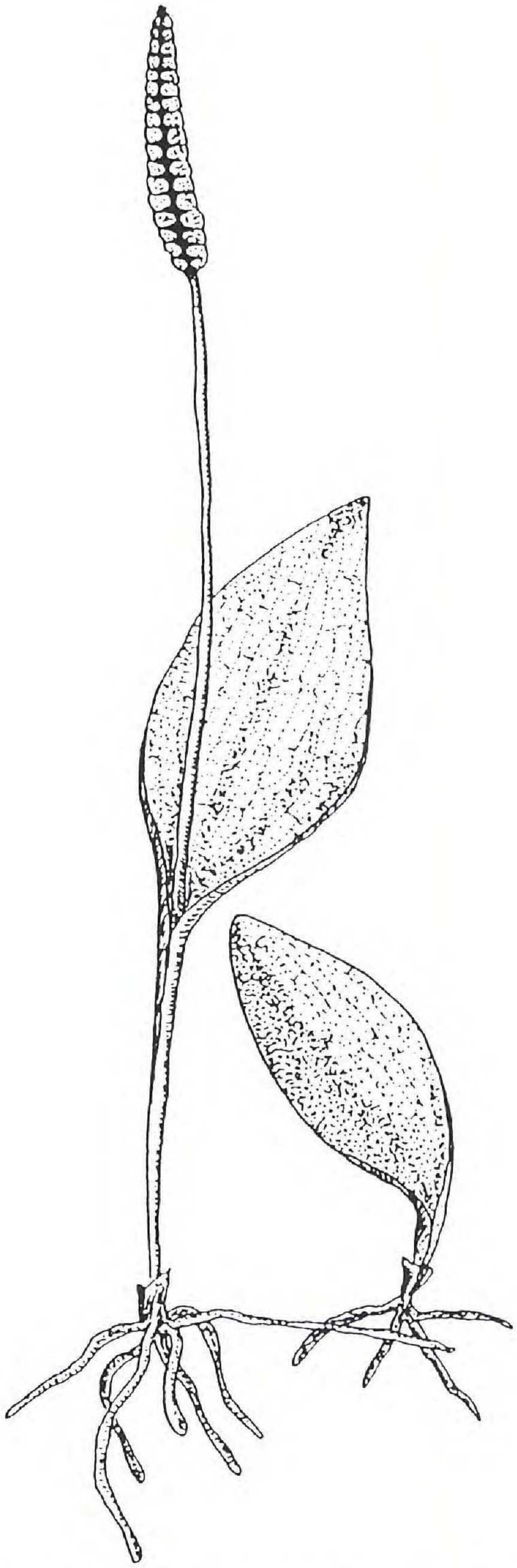

Plate 1. *Ophioglossum vulgatum* var. *pseudopodum* (Northern Adder's-Tongue Fern), natural size. Illustration by Susan Alix Williams, reproduced with the permission of the artist. Originally published in Susan Alix Williams, *Wildflowers of Rowe,* 1991, published by the Rowe Historical Society.

Ophioglossum is characterized by extremely large chromosome numbers. *Ophioglossum reticulatum* L., with n = 631, is believed to possess the largest number of chromosomes of any organism known. Other *Ophioglossum* species range from n = 120 to n = 566 (Tryon and Tryon, 1982).

Ophioglossum vulgatum L. is typical of its genus. The single sterile leaf is elliptic, 2.5–10 cm long and 1–4 cm wide, with a tapered base and reticulate venation. The stalk of the fertile spike is 4–23 cm long with sporangia arranged in two parallel rows 1.5–5 cm long at the tip. From the erect rhizome, numerous slender roots diverge. The gametophyte is subterranean and mycoparasitic (Wagner et al., 1985; Goswami, 1987). The roots of *O. vulgatum*, like those of the other members of the Ophioglossales, are hairless, and some botanists have assumed that mycorrhizae must play an important role in the uptake and transport of water and nutrients (Wagner et al., 1985). Khandelwal (1990) has suggested a chromosome number for *O. vulgatum* of n = 120.

Ophioglossum vulgatum now occurs in North America, Europe, and Asia (Clausen, 1938). This wide range has led botanists to conclude that *O. vulgatum* itself is ancient and was probably widely dispersed in Laurasia before the separation of North America from Eurasia. While it normally occurs at lower elevations, specimens of *O. vulgatum* in the Gray Herbarium have been collected at 4500 feet in Nepal and at 10,000 feet in China.

Fernald (1939) proposed two North American varieties of *Ophioglossum vulgatum*: var. *pseudopodum*, the northern adder's-tongue fern, and var. *pycnostichum*, the southeastern adder's-tongue fern. *Ophioglossum vulgatum* var. *pseudopodum* has a pale green sterile blade that tapers at the base. *Ophioglossum vulgatum* var. *pycnostichum* Fern. is distinguished by its dark green sterile blade with a rounded base (Fernald, 1950). Wagner (1971) believed spore size was another differentiating characteristic of these two varieties, the spores of *O. vulgatum* var. *pseudopodum* averaging 20% larger than those of *O. vulgatum* var. *pycnostichum*. McAlpin (1971) argued that the presence of a persistent sheath in var. *pycnostichum* and its absence in var. *pseudopodum* was an important taxonomic character for distinguishing the two varieties. Lellinger (1985) argued that the two varieties are sufficiently distinct and sufficiently different from *O. vulgatum* L. to justify two separate North American species, *O. pusillum* Raf. and *O. pycnostichum* (Fern.) Löve & Löve. More recently Wagner and Wagner recognized Rafinesque's *O. pusillum* as the northern

form and included *O. pycnostichum* (Fern.) Löve & Löve within the Linnaean *O. vulgatum* (Wagner and Wagner, 1993).

The present study follows the broader and more traditional treatment of *O. vulgatum*. However, all plants investigated represent Rafinesque's *O. pusillum* and Fernald's *O. vulgatum* var. *pseudopodum* (Blake) Farw. and are referred to as *O. vulgatum*.

Life History

Spore Development, Dispersal and Germination

Sori of *Ophioglossum vulgatum* are produced on the single fertile sporophyll with spores maturing in late July or early August in New England. The fully mature sporangia dehisce to release a cloud of yellow, dustlike haploid spores. No estimates have been made of spore production in *O. vulgatum*; however, other ferns with spores of similar size produce between 7.5×10^5 and 7.5×10^9 spores per fertile frond (Page, 1979).

Fern spores are often carried long distances by air currents. However, an increase in atmospheric moisture may result in a sudden, local descent of spores in rainstorms and hence peaks of fern spore deposition may occur during the first rainfall following a warm, dry spell (Page, 1979).

After landing, the spores of *Ophioglossum vulgatum* often percolate several centimeters through porous soil before reaching a stable substrate (Edwards, 1982; Wagner et al., 1985). Germination may commence immediately and proceed rapidly if conditions are suitable; otherwise, development may extend over one or more years (Foster and Gifford, 1974) since prothalli are capable of overwintering (Page, 1979). The spores may also remain dormant for extended periods (Goswami, 1987). When the gametangia mature, motile sperms are released and may move several centimeters either by mass flow of ground water or by flagellar motion (Wagner et al., 1985).

Since the gametophytes of *Ophioglossum* and *Botrychium* lack chloroplasts and grow beneath the soil surface, intragametophytic selfing may be favored because it is unlikely that two spores will land sufficiently near to one another for cross-fertilization to occur and soil particles may impede sperm (Klekowski and Baker, 1966; Tryon and Tryon, 1982; Peck et al., 1990).

Wagner et al. (1985), however, contend that species with subterranean gametophytes may cross-fertilize as frequently as spe-

cies with surficial gametophytes because of the crowding of gametophytes that results when "spore showers" settle out. In addition, herbivores have been observed browsing sporangial clusters and Wagner et al. (1985) hypothesize that spores of *O. vulgatum* may resist digestive processes, be deposited in feces, and germinate in clusters (Wagner et al., 1985).

Nonetheless, isozyme electrophoresis of various *Botrychium* species suggests that intragametophytic selfing predominates (Soltis and Soltis, 1986; McCauley et al., 1985; Watano and Sahashi, 1992).

Vegetative Reproduction

Ophioglossum vulgatum frequently reproduces vegetatively through sporophytes arising directly from the roots of parent plants (Foster and Gifford, 1974). Edwards (1982) studied vegetative growth in a population of *O. vulgatum* in southern England and traced the root system of a single plant for 1.8 m at a depth of 18–25 cm in the soil. Two clonal sporophytes were produced within this distance and the roots may have been connected with other more distant fronds.

Ecology of *Ophioglossum vulgatum*

Ophioglossum vulgatum has been found in such diverse sites as bogs, fens, damp sands, pastures, wet meadows, grassy swales, moist woods, rich swamplands, mud creeks, and cedar swamps. Occasionally it occurs on dry, sandy beaches (Clausen, 1938) or hillsides (SCHN, B. A. Phelps and A. V. Osmun, Cornwall Bridge, CT, 1902) and the subterranean gametophyte may be an adaptation to seasonal drying and/or fire (Wagner et al., 1985).

Overall, *Ophioglossum vulgatum* favors early successional sites (Coddington and Field, 1978; Tryon and Tryon, 1982) and its occurrence often appears to be related to disturbance such as the disruption of soil by grazing herbivores.

METHODS

Herbarium Research and Field Study

In order to determine historical and/or present sites, all Massachusetts specimens of *Ophioglossum vulgatum* were examined

at the Smith College Herbarium (SCHN), the Herbarium of the University of Massachusetts–Amherst (MASS), the Gray Herbarium (GH), the New England Botanical Club Herbarium (NEBC), and the Yale University Herbarium (Y). In addition, the collection records of Harry Ahles and Roberta Poland, both of whom collected extensively in Franklin County, were examined at MASS. The Massachusetts Natural Heritage Program of the Massachusetts Division of Fisheries and Wildlife (MNHP) in Boston also provided records of present and historical populations.

Since 1961, *Ophioglossum vulgatum* has been recorded in Massachusetts at 13 sites. Two sites were not visited due to difficulties in gaining access, although MNHP records indicate that other workers have recently sought *O. vulgatum* at both of these sites without success. The remaining eleven Massachusetts sites were visited between June 1 and August 30, 1992.

At each site where *Ophioglossum vulgatum* was relocated, every sporophyte was flagged and recorded on a detailed site map. The reproductive status of the sporophyte, whether sterile or fertile, was also recorded. Because all populations occurred in open areas surrounded by forest, three species lists were compiled for each site: (1) the dominant trees of the surrounding forest, (2) all vascular plant species in the area where *O. vulgatum* occurred, and (3) associated species, i.e., those within 1 m of any specimens of the population of *O. vulgatum*. Nomenclature of vascular plant species follows Fernald (1950) unless otherwise noted.

Property owners, conservation agents and other researchers were consulted to determine the history of each property, including disturbances caused by beaver occupation, logging, fires, agricultural practices and applications of herbicides.

Laboratory Investigations

Starch gel electrophoresis was carried out on tissue samples from four of the five Massachusetts sites where *Ophioglossum vulgatum* occurred after permission to collect this threatened species was obtained from MNHP. Because of the very small size of its *O. vulgatum* population, no live material was collected at the Brewster site. Because of the large size of the population and the clustered occurrence of the sporophytes at the Conway site, live material was taken from 108 sporophytes, 12 from each of 7 clusters distributed around the site and 24 taken randomly from

around the site. At each of the remaining three Massachusetts field sites, leaf segments were collected from about 12 sporophytes. For purposes of comparison, live material was also obtained from a site at Mt. Sunapee, New Hampshire. Segments ranged from 40 mg to 100 mg depending on the size of the blade. Care was taken not to uproot or otherwise disturb plants from which leaf segments were taken. Each leaf segment was assigned a number and placed in a small plastic bag, its location recorded on a map of the site.

Live material was placed on ice immediately and kept on ice from a few hours to a maximum of five days before processing in the laboratory. Except where noted, the protocol for electrophoresis of ferns by Soltis et al. (1983) was followed.

In the laboratory each specimen was weighed and placed in a mortar chilled in ice. The phosphate grinding buffer-polyvinylpyrrolidone (PVP) solution was prepared in advance except for 2-mercaptoethanol which was added immediately before grinding. The solution was then added to the mortar in the ratio of .4 ml to 100 mg of live material and the mixture ground into a homogenate. Filter paper wicks with dimensions 5 mm × 7 mm were saturated with homogenate, then placed in labeled microtubules. In many instances wicks were utilized immediately. Any wicks held longer than 4 hours were placed at −80°C until immediately before use, a maximum of 15 weeks.

Starch gels with dimensions 13.25 cm × 24.75 cm × 7.5 mm were prepared using 30 g of potato starch (SIGMA S-4501), 50 g of sucrose and 400 ml of gel buffer. Normally, the solution was heated for about 1.5 minutes after reaching the gel point, aspirated for 1 minute, then poured into gel forms, wrapped in plastic wrap, and refrigerated overnight. With gel buffer #6 (Soltis et al., 1983) the solution was heated for 1.75 minutes, then aspirated for 1.5 minutes.

Microtubules containing wicks to be run were thawed in ice water for approximately 15 minutes. Wicks were removed from the microtubule and inserted in one of twenty-four wells along the center of each gel.

Gels were run under refrigeration at approximately 4°C for from 3 to 5 hours with a current between 40 and 65 milliamperes. All enzymes migrated anodally. Two bags of crushed ice and water were placed on top of each gel to prevent overheating and were

changed as needed. When a gel was finished, the power was turned down to 10 milliamperes until ready for slicing. Three to four slices approximately 1.9 mm thick were made from each gel.

One hundred ml of stain was placed on each gel slice which was then kept in the dark at room temperature for from 1 hour to 21 hours depending on the enzyme. Gels were scored and some photographed. Gel slices were then labeled, wrapped in plastic and refrigerated for future reference.

An effort was made to develop a protocol for electrophoresis of *Ophioglossum vulgatum* for each of the following enzymes: Acid phosphatase (APH, EC 3.1.3.2), Aconitase (ACON, EC 4.2.1.3), Aldolase (ALD, EC 4.1.2.13), Aspartate aminotransferase (AAT, EC 2.6.1.1), Catalase (CAT, EC 1.11.1.6), Esterase (EST, EC 3.1.1.–), Fructose-1,6-diphosphatase (F1,6DP, EC 3.1.3.11), Glutamate dehydrogenase (GDH, EC 1.4.1.2), Glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), Glyceraldehyde-3-phosphate dehydrogenase (G3PD, EC 1.2.1.12), Hexokinase (HK, EC 2.7.1.1), Isocitrate dehydrogenase (IDH, EC 1.1.1.42), Malate dehydrogenase (MDH, EC 1.1.1.37), Malic enzyme (ME, EC 1.1.1.40), 6-Phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), Phosphoglucoisomerase (PGI, EC 5.3.1.9), Phosphoglucomutase (PGM, EC 2.7.5.1), Shikimate dehydrogenase (SkDH, EC 1.1.1.25), and Triosephosphate isomerase (TPI, EC 5.3.1.1).

Protocols were developed that produced observable bands for ten enzymes and 26 loci:

1. Acid phosphatase (APH-1, APH-2, APH-3)
2. Esterase (EST-1, EST-2, EST-3)
3. Glucose-6-phosphate dehydrogenase (G6PD-1, G6PD-2, G6PD-3)
4. Isocitrate dehydrogenase (IDH)
5. Malate dehydrogenase (MDH-1, MDH-2, MDH-3)
6. Malic enzyme (ME-1, ME-2, ME-3)
7. 6-Phosphogluconate dehydrogenase (6PGD-1, 6PGD-2)
8. Phosphoglucoisomerase (PGI-1, PGI-2)
9. Phosphoglucomutase (PGM-1, PGM-2)
10. Triosephosphate isomerase (TPI-1, TPI-2, TPI-3, TPI-4).

For G6PD, IDH, MDH, 6PGD and PGM, buffer system 2 was utilized. For APH and ME, buffer system 6 was used. For EST,

PGI and TPI, buffer system 8 was used. When more than one locus was observed for an enzyme, loci were numbered with the locus migrating farthest identified as number 1.

A set of gels was run for each of the seven clusters of sporophytes in the Conway site in order to assess genetic variability within clusters. Another set of gels was then run with one or two specimens from each of the seven Conway clusters to assess variability between clusters. A third set of gels was run using twelve sporophytes from around the Conway site outside of the clusters already sampled. A fourth set of gels was run for each of the other five populations using a Conway specimen as a standard. Finally, a set of gels was run with representatives of each of the populations to assess variation between populations. Included on this final gel were specimens of a tropical species, *Ophioglossum reticulatum*, obtained from the Lyman Plant House at Smith College, and specimens of *Botrychium dissectum* and *B. dissectum* var. *obliquum* obtained from a local woodland. The same Conway specimen was used as a standard.

RESULTS AND DISCUSSION

Ophioglossum vulgatum in Massachusetts: Historical Status

The earliest reference to *Ophioglossum vulgatum* in Massachusetts appears in Josselyn's *New England's Rarities* (1672), where the species is reported to occur "upon dry hill grounds [and] in places where the water hath flood all Winter. . . ."

Ophioglossum vulgatum is listed in Hitchcock's *Catalogue of Plants Growing Without Cultivation in the Vicinity of Amherst College* (1829) and in the first complete flora of Massachusetts (Hitchcock, 1833) with a location "in the vicinity of Amherst."

MNHP lists 105 current or historical records for *Ophioglossum vulgatum*. Collection dates range from 1848 to 1991 from 89 cities and towns in all 14 counties (Figure 1). The five herbaria consulted for this project contain 101 dated Massachusetts specimens from 70 cities and towns in all 14 counties. Collection dates range from 1848 to 1986. *Ophioglossum vulgatum* was most widely distributed in Massachusetts from 1870 to 1939 and more widely collected in western Massachusetts (Table 1). Collections in eastern Massachusetts peaked during the period 1870 to 1919. Only five

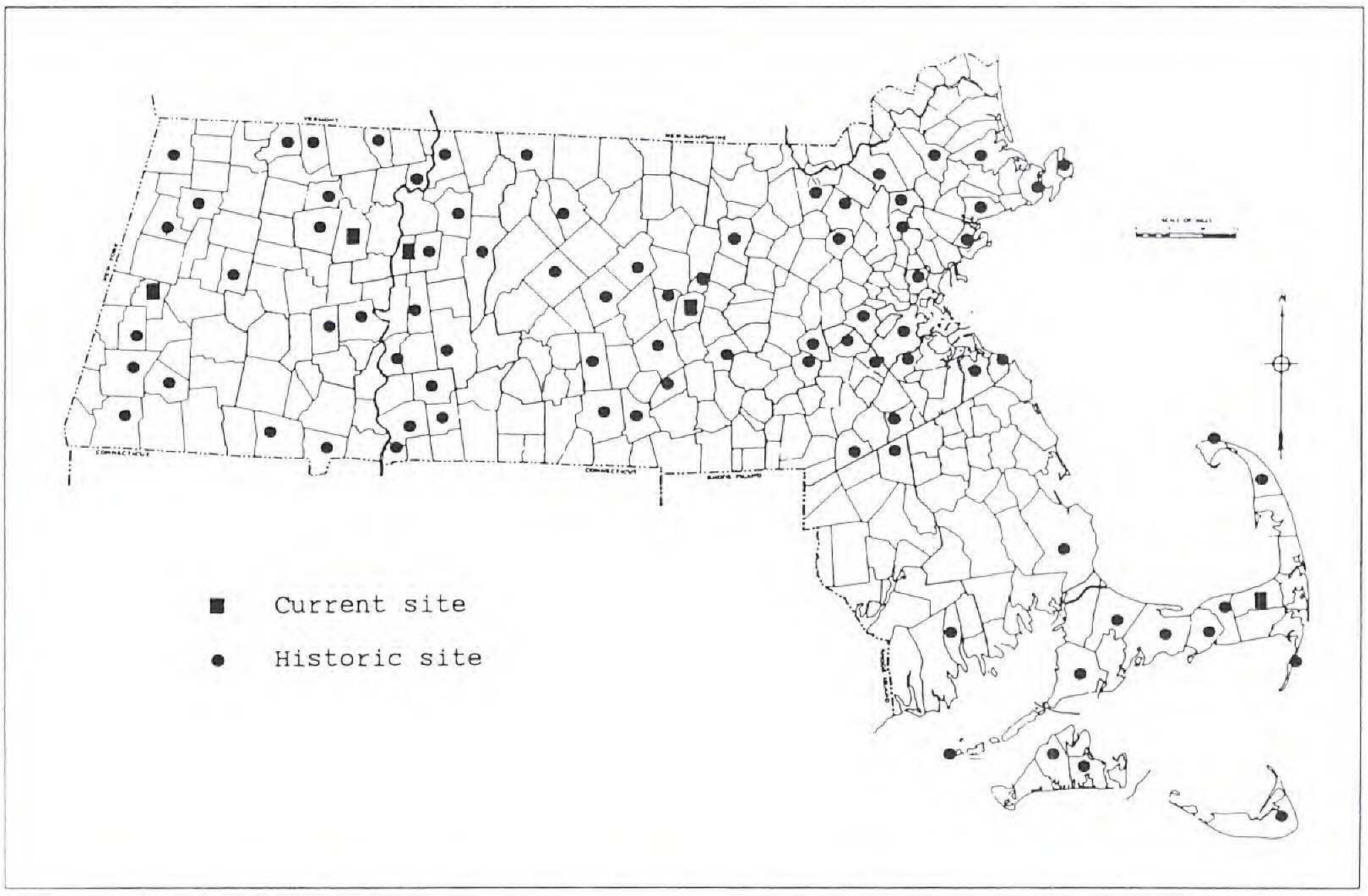

Figure 1. Current distribution and recorded historical sites of *Ophioglossum vulgatum* by city or town in Massachusetts.

specimens have been collected east of Worcester County since 1919. No more than 14 collections exist for any decade and in some decades *O. vulgatum* has not been collected at all.

Coddington and Field (1978) considered *Ophioglossum vulgatum* widespread but rare in Massachusetts and recommended its inclusion in a proposed list of rare and endangered plant species for the state, noting ". . . few field botanists have seen it. Characteristic of early successional habitats." Massachusetts collections of *O. vulgatum* range from sea level to 1200 feet, in habitats ranging from bogs, abandoned beaver meadows and pastures to old fields and power line rights-of-way.

Field Sites and Population Histories

Current Sites in Massachusetts

Five populations of *Ophioglossum vulgatum* were relocated, one each in Conway and Sunderland (Franklin County), Lenox (Berkshire County), Boylston (Worcester County), and Brewster (Barnstable County).

Conway, MA. The Conway, MA site is located northwest of Conway State Forest in Franklin County. It occupies .96 ha at an

Table 1. Dated records of *Ophioglossum vulgatum* from Mass. Natural Heritage Program; records for eastern and western Massachusetts grouped by decade. Eastern Massachusetts includes Essex, Middlesex, Suffolk, Norfolk, Bristol, Plymouth, Barnstable, Dukes and Nantucket Counties. Western Massachusetts includes Berkshire, Hampden, Hampshire, Franklin and Worcester Counties.

Time Period	Eastern Mass.	Western Mass.	Total
1840–1849	0	1	1
1850–1859	0	0	0
1860–1869	0	2	2
1870–1879	9	5	14
1880–1889	9	4	13
1890–1899	2	1	3
1900–1909	5	7	12
1910–1919	10	4	14
1920–1929	2	12	14
1930–1939	0	9	9
1940–1949	0	1	1
1950–1959	0	0	0
1960–1969	1	1	2
1970–1979	1	2	3
1980–1989	1	2	3
1990–present	0	0	0
Total	40	51	91

elevation of 450 m above sea level and slopes gently from southwest to northeast.

Aerial photographs indicate that low shrub vegetation covered the site in 1952 and 1958. Beavers were active in the area in the early 1960's (C. J. Burk, pers. comm.) and by the mid-1960's the site was covered with water to a depth of over 1 m. The pond was abandoned in the late 1960's, probably as a result of trapping. Aerial photographs taken in 1971 show that half of the site was covered with open water and the rest with grass and sedge vegetation. By July, 1980, the open water occurred only in front of the dam (Mosher, 1981). Standing water now covers much of the site at frequent intervals during the growing season.

Dominants of the surrounding forest are typical components of the northern hemlock-hardwood forest including *Acer saccharum, Betula lutea, Fagus grandifolia, Pinus strobus* and *Tsuga canadensis.*

The current Conway population was first discovered by Nancy D. Mosher (Nancy D. McMaster) in 1980 (Mosher, 1981) and

Plate 2. Abandoned beaver meadow in Conway (Franklin County), MA, an open marsh dominated by *Typha latifolia, Spiraea latifolia* and *S. tomentosa* and a current site for *Ophioglossum vulgatum.*

reported to MNHP in 1985. In July, 1980, when the vegetation in the site was first sampled, 26 sporophytes of *Ophioglossum vulgatum* were present (Mosher, 1981). Approximately 40 sporophytes of *O. vulgatum* were found in the site in 1985 (N. McMaster, 1989) and approximately 50 in 1990 (N. McMaster, 1990, unpubl. data). By July, 1991, the population had increased sharply to a total of 901 sporophytes (R. McMaster, 1991), and by August and September, 1992, had declined slightly to 866 sporophytes.

Most sporophytes in 1991 occurred in clusters of 10–27 stems. Clusters were roughly circular with a radius of about .5 m. Frequently, clusters grew on patches of somewhat elevated substrate, either around the bases of shrubs such as *Alnus rugosa, Salix* spp., or *Spiraea* spp., or on submerged logs covered with bryophytes in the wetter soils of depressions. A few clusters occurred in lower areas, however, and some sporophytes were not clustered, with individual stems occurring .5 m or more from the nearest member of the population. An estimated 61% of the sporophytes were fertile (R. McMaster, 1991).

Ophioglossum vulgatum was not found where open water or mud was present, nor in drier areas around the margins where trees and shrubs predominated. Its frequency decreased abruptly

on the southwestern edge of the site which supported a dense cover of sedges and grasses.

In the immediate vicinity of the *Ophioglossum vulgatum* population, associated shrubs and small trees included *Acer rubrum, Alnus rugosa, Betula populifolia, Pinus strobus, Salix discolor, S. sericea, Spiraea latifolia* and *S. tomentosa*. Associated herbaceous species in the immediate vicinity included *Carex lurida, Drosera rotundifolia, Dulichium arundinaceum, Eleocharis tenuis, Epilobium strictum, Equisetum arvense, Eupatorium maculatum, E. perfoliatum, Galium tinctorium, Glyceria canadensis, Hydrocotyle americana, Hypericum virginicum, Impatiens capensis, Juncus effusus, Juncus* sp., *Leersia oryzoides, Liparis loeselii, Lycopus virginicus, Mentha arvensis, Muhlenbergia mexicana, Onoclea sensibilis, Osmunda regalis, Polygonum sagittatum, Sagittaria latifolia, Scirpus cyperinus, Solidago canadensis, S. graminifolia, Sparganium americanum, Thelypteris palustris* Schott, *Typha latifolia,* and *Viola pallens* (McMaster, 1991).

Sunderland, MA. The Sunderland, MA (Franklin County) site is located in an electrical transmission line right-of-way at an elevation of 220 m. The site slopes gradually from east to west and is seasonally wet.

The right-of-way was established in about 1912 and is maintained by cutting, pruning and periodic chemical treatment of any trees or shrubs that might interfere with the operation of the transmission lines. The site was last cut back or treated in 1989 (R. Farrell, pers. comm.).

On both sides the site adjoins northern hardwood forest dominated by *Acer saccharum, Betula papyrifera, B. populifolia, Carya ovata, Pinus strobus,* and *Quercus rubra.*

The population of *Ophioglossum vulgatum* was first reported in 1986 by Bruce Sorrie of the MNHP and Robert Ruhful of the University of Massachusetts–Amherst. A total of 80 plants of *O. vulgatum* were located over a .4–.8 hectare area.

At this site in August, 1992, the population of *Ophioglossum vulgatum* had decreased to 21 sporophytes occurring throughout a roughly rectangular area 8 m × 2 m. There were only 2 fertile sporophytes. Most leaf blades were small with atypically brown or yellow patches. Associated shrubs and small trees in the immediate vicinity included *Acer rubrum, Betula lenta, Cornus racemosa, Hamamelis virginiana, Lindera benzoin, Lyonia ligustrina, Rubus* sp. and *Salix* sp. Associated herbaceous species

included *Achillea millefolium, Carex scoparia, C. stipata, Cirsium arvense, Equisetum sylvaticum, Fragaria virginiana, Galium asprellum, Geranium maculatum, Hypericum ellipticum, Juncus effusus, Onoclea sensibilis, Osmunda cinnamomea, Platanthera flava* (L.) Lindl., *Polygonum sagittatum, Prunella vulgaris, Solidago graminifolia, Thelypteris palustris,* and *Viola* spp.

Lenox, MA. The Lenox, MA (Berkshire County) site is located on the edge of an open meadow at an elevation of 370 m. The seasonally wet site slopes gently from west to east and has been described as a "sloping graminoid fen" (P. Weatherbee, pers. comm.).

The area was used for pasture until 1939 and aerial photographs show it has been kept open, probably by mowing or haying, since. During the 1980's a stream to the west was dammed by beavers, causing surface water to flow across the site, which was last mowed in 1989 (P. Weatherbee, pers. comm.).

The forest surrounding the meadow is dominated by *Acer rubrum, A. saccharum, Carya ovata, Malus* sp., *Pinus strobus* and *Populus tremuloides* with an understory of *Betula papyrifera, Fraxinus americana, Prunus virginiana,* and *Viburnum recognitum. Celastrus orbiculatus* and *Vitis* spp. frequently overtop the canopy to the west. *Alnus* sp. dominates the wetter eastern boundary of the meadow.

The population of *Ophioglossum vulgatum* was discovered and reported to MNHP by Pamela B. Weatherbee in 1991. A total of 205 sporophytes occurred in a roughly rectangular area measuring 11 m × 26 m. Twenty-two percent of the sporophytes were fertile.

Within this area in August, 1992, the population of *Ophioglossum vulgatum* had declined to 62 sporophytes. Approximately 33% of these were fertile. Most were growing beneath low shrubs, either *Cornus stolonifera, Salix serissima* or *Salix discolor.* Other associated species in the shrub layer included *Potentilla fruticosa, Viburnum recognitum, Rhamnus cathartica, Salix serissima, S. discolor* and *Vitis* sp. Herbaceous species included *Achillea millefolium, Carex stricta* var. *strictior, Equisetum arvense, Eupatorium maculatum, Festuca* sp., *Fragaria virginiana, Galium triflorum, Geum rivale, Juncus canadensis, J. dudleyi, Lysimachia ciliata, Onoclea sensibilis, Parthenocissus quinquefolia, Phleum pratense, Prunella vulgaris, Rudbeckia hirta, Scirpus atrovirens, Spiraea latifolia, Thelypteris palustris, Trifolium repens, T. agrarium,* and *Valeriana officinalis.*

Plate 3. Two sporophytes of *Ophioglossum vulgatum*, one with sporophyll, in Lenox (Berkshire County), MA.

Boylston, MA. The Boylston, MA (Worcester County) site is located at the eastern edge of a meadow which slopes from west to east at an elevation of 170 m. Occasionally, shallow standing water occurs on the lowest part of the meadow about 50 m north of the site (J. Wright, pers. comm.).

The site was used for pasture before 1986. The meadow was cut for hay in 1986 and 1987 and mowed once a year in October in 1988, 1989, 1990 and 1992.

The adjacent forest includes a canopy of *Acer rubrum, Carya* sp., *Fraxinus americana, Malus* sp., *Pinus strobus, Prunus serotina* and *Quercus velutina* and an understory of *Prunus virginiana* and *Rhamnus frangula.*

Shrubs and small trees found in the adjacent upland meadow include *Acer rubrum, Cornus amomum, Prunus serotina, Rhamnus frangula, Salix bebbiana, Spiraea latifolia, S. tomentosa,* and *Viburnum recognitum.* Herbaceous species of the upland meadow include *Daucus carota, Eupatorium maculatum, Galium tinctorium, Onoclea sensibilis, Rhus radicans, Rosa nitida, Solidago canadensis, S. rugosa, Trifolium hybridum, T. pratense, Vicia cracca* and *Vitis labrusca.*

The "very vigorous" population of *Ophioglossum vulgatum* consisted of 30–40 plants when first reported in 1989 by Marc Larocque. When the population was surveyed in August, 1992, it had increased to 109 sporophytes located in a level area about 12 m × 20 m. However, only 18% of the sporophytes were fertile. Many sporophytes occurred beneath shrubs of *Cornus amomum* and *Salix* spp. between .3 m and 1.0 m high. Associated shrubs and tree seedlings included *Acer rubrum, Alnus rugosa, Betula populifolia, Cornus amomum, Malus* sp., *Prunus serotina, Quercus rubra, Rhamnus frangula, Rhus radicans, Rosa nitida, Spiraea latifolia, S. tomentosa, Vaccinium corymbosum,* and *Viburnum recognitum.* Herbaceous species included *Achillea millefolium, Agrostis stolonifera, Asclepias syriaca, Asparagus officinalis, Carex stricta, Daucus carota, Galium tinctorium, Onoclea sensibilis, Osmunda regalis, Oxalis montana, Solidago canadensis, S. gigantea, S. rugosa, Trifolium hybrida, T. pratense, Rubus* sp., and *Vicia cracca.*

Brewster, MA. The Brewster, MA (Barnstable County) site is located in a small clearing adjacent to a pond at an elevation of 8 m. The site is seasonally wet and was probably kept clear by

periodic mowing until 1985. There has been no clearing since 1985 (R. Finch, pers. comm.).

The adjacent woodland is dominated by *Acer rubrum* and *Nyssa sylvatica.*

The population of *Ophioglossum vulgatum* included 19 plants when first reported by Richard LeBlond in 1985. Mario Di-Gregorio, then Conservation Agent for the Town of Brewster, observed the population from 1986 to 1989 and reported finding six sterile plants and four to five fertile plants each year. He had been unable to relocate any plants since 1989.

In July, 1992, only a single sterile sporophyte was located. Associated shrubs and small trees included *Acer rubrum, Leucothoë racemosa, Lyonia ligustrina, Nyssa sylvatica, Rhododendron viscosum, Vaccinium corymbosum* and *V. macrocarpon.* Herbaceous species included *Maianthemum canadense, Rubus hispidus,* and *Trientalis borealis.* Extensive areas were covered by *Sphagnum* spp. and other mosses.

Recent Historical Sites in Massachusetts

Six historical Massachusetts sites were visited but no population of *Ophioglossum vulgatum* found. These occurred at Lanesboro (Berkshire County), Rowe and Gill (Franklin County), Northampton (Hampshire County), Plymouth (Plymouth County), and Falmouth (Barnstable County).

Lanesboro, MA. The Lanesboro, MA population of *Ophioglossum vulgatum* was located by Pamela B. Weatherbee in 1984 when 15 sterile and 3 fertile sporophytes were found at the eastern base of an outcrop of rock in an old field, an area underlain by limestone. No sporophytes have been found on the site since 1984 (P. Weatherbee, pers. comm.).

Rowe, MA. The Rowe, MA population was located by Nancy Williams and Susan Williams in 1983 when approximately 150 sporophytes were found in a "borrow pit." In 1973 sand and gravel were removed from the site which was subsequently colonized by herbaceous vegetation, small shrubs and tree seedlings. The population of *Ophioglossum vulgatum* was seen yearly from 1983 to 1988 but not since in spite of repeated observations of the area (N. Williams, pers. comm.).

Gill, MA. The Gill, MA population was found in 1961 in a "marshy field." I was unable to relocate the population in June,

1992. The wet meadow apparently had been disturbed recently by mowing and excavation.

Northampton, MA. The Northampton, MA population was located by Harry Ahles in 1978 in what was described as a wet hay field. Bruce Sorrie searched unsuccessfully for the population in 1986. I was unable to relocate the population in July, 1992.

Plymouth, MA. The Plymouth, MA population was located by Bruce Sorrie in 1983 in an acid seep. I was unable to relocate the population in June, 1992.

Falmouth, MA. The Falmouth, MA population was located by H. K. Svenson in 1971 in the bottom of a glacial kettle near the town landfill. The site was apparently destroyed in the expansion of the landfill and my efforts to relocate the population in June, 1991 were unsuccessful.

1993 Observations

All four western Massachusetts populations of *Ophioglossum vulgatum* that were extant in 1992 were monitored during the hot, dry summer of 1993. The Conway population was thriving throughout the summer of 1993. Stem counts in eight permanent plots showed a 64% increase over similar counts made in 1991. The Sunderland population had dwindled to one small, withered blade when observed on August 5, 1993. No sporophytes of the Lenox population were in evidence on July 24, 1993. Some sporophytes of the Boylston population were observed to be in good condition in early July, 1993, but had died back by August 18, 1993 (J. Wright, pers. comm.).

Laboratory Investigations

A total of 157 individuals of *Ophioglossum vulgatum* was assayed at 26 loci (Table 2). All individuals in all populations were found to be homozygous and monomorphic for all observable loci.

Additional confirmation of the observed monomorphism among the specimens assayed was obtained by electrophorescing material from all five populations of *Ophioglossum vulgatum* (four from Massachusetts, one from New Hampshire) with material of *O. reticulatum, Botrychium dissectum* and *B. dissectum* var. *obliquum* on a single gel. Results for the three New Hampshire spec-

Table 2. Total sample size and number of specimens for which satisfactory band resolution was achieved at each locus in each site, CONway, SUNderland, LENox, BOYlston and MT. Sunapee. (—) indicates unsatisfactory band resolution.

Locus	CON	SUN	LEN	BOY	MTS	TOTAL
Total sample size	108	13	11	13	12	157
APH-1	59	—	—	—	—	59
APH-2	33	—	—	—	—	33
APH-3	24	—	—	—	—	24
EST-1	16	—	—	—	—	16
EST-2	12	—	—	—	—	12
EST-3	12	—	—	—	—	12
G6PD-1	59	7	7	11	1	85
G6PD-2	52	—	—	—	—	52
G6PD-3	16	—	—	—	—	16
IDH	75	13	11	1	12	115
MDH-1	85	13	11	11	8	128
MDH-2	79	12	11	11	3	116
MDH-3	21	—	—	—	—	21
ME-1	85	13	5	1	—	104
ME-2	38	13	5	1	—	57
ME-3	1	1	5	3	—	10
PGI-1	93	13	11	11	12	140
PGI-2	69	13	11	10	12	115
PGM-1	77	13	11	13	8	122
PGM-2	29	—	—	—	—	29
6PGD-1	72	—	3	—	—	75
6PGD-2	50	—	—	—	—	50
TPI-1	39	1	11	1	7	59
TPI-2	22	1	11	2	7	43
TPI-3	19	1	7	—	7	34
TPI-4	6	—	—	—	7	13

imens of *O. vulgatum* were inconclusive and warrant additional study. Bandings for *O. reticulatum*, *B. dissectum* and *B. dissectum* var. *obliquum* were distinct from each other and from *O. vulgatum*.

The number of loci assayed represents only a small portion of the total genome of *Ophioglossum vulgatum*. The significance of complete monomorphism for these loci, however, is enhanced by the fact that the same 26 loci found to be monomorphic for specimens of *O. vulgatum* have been widely assayed for other homosporous ferns and frequently have been observed to be polymorphic (McCauley et al., 1985; Soltis and Soltis, 1986; Soltis and Soltis, 1988; Watano and Sahashi, 1992). No other published data on genetic variation in *Ophioglossum vulgatum* was found.

Lack of genetic variability often has been cited as a factor in the decline in population size and, in some cases, in the extinction of rare plant species (Frankel and Soulé, 1981; Simberloff, 1986; Barrett and Kohn, 1991; Huenneke, 1991). Data from the electrophoresis of specimens from four of the Massachusetts populations of *Ophioglossum vulgatum* show a complete absence of genetic variability at all loci assayed. Any explanation for this observation must account for three phenomena: (1) the apparent lack of genetic variability within sporophytes; (2) the apparent lack of genetic variability among individuals within each population; and (3) the apparent lack of genetic variability among populations.

If intragametophytic selfing occurs, all heterozygosity will be lost in one generation but there still may be some differentiation within a population. Reductions in genetic variability may also occur as a result of small effective population size. Decline in effective population size may result when few individuals reproduce, when the number of progeny produced by individuals varies widely, and/or when a population bottleneck, an extreme reduction in effective population size, occurs resulting in the transmission of only a small subset of the total genetic variability of the population to the next generation (Frankel and Soulé, 1981). A population with a small effective population size is also susceptible to further loss of alleles due to genetic drift. Edwards (1982) observed a population of *Ophioglossum vulgatum* in southern England, for example, in which only 1–2% of the sporophytes bore fertile fronds. Similarly, in the Sunderland population less than 10% of the sporophytes found in the summer of 1992 had fertile fronds. In addition, populations of clonal species such as *O. vulgatum* may contain numerous sporophytes of a single genotype.

New populations established by dispersal of spores from a genetically monomorphic population will normally be of the same homozygous genotype. If the species is capable of rapid vegetative growth, it is possible for a number of large, homozygous, monomorphic populations to develop in a relatively short period of time.

One adaptive compensation for the loss of genetic variation in a population may be the purging of deleterious recessive alleles through repeated episodes of in-breeding (Frankel and Soulé, 1981; Lesica and Allendorf, 1992). By this mechanism, a predominantly self-fertilizing species such as *Ophioglossum vulgatum* character-

ized by low genetic variability may have evolved a complex of alleles co-adapted to a very specific set of environmental conditions. While it is unable to adapt successfully to changes in those conditions, it is suited to sites where those conditions prevail.

The four western Massachusetts populations may have arisen from a single monomorphic population. Gene flow from genetically distinct populations apparently has not occurred or has occurred at sufficiently low frequencies that any variability introduced has been lost through repeated intragametophytic selfing, population bottlenecks and drift. Electrophoretic analysis of populations of *Ophioglossum vulgatum* in neighboring states could shed additional light on these questions.

CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

The five current Massachusetts sites for *Ophioglossum vulgatum* vary significantly in geography, climate, soil and surrounding forest type, but share three important characteristics:

(1) Each site has a history of disturbance that has reduced competition from shrubs and small trees. The Conway site was occupied by beavers. The Sunderland site has been subject to periodic pruning or application of herbicide for maintenance of the power line right-of-way. The Lenox site was occupied by beavers and has been mowed occasionally. The Boylston site was formerly pasture and has been mowed or hayed regularly for many years. The Brewster site was probably maintained irregularly as a lawn.

(2) Each site is characterized by wet conditions for at least a part of the year. The Conway site is saturated throughout the growing season with standing water at frequent intervals. The Sunderland, Lenox, Boylston, and Brewster sites are subject to seasonal flooding, usually in spring and fall.

(3) Each site supports a characteristic flora. Although no single plant species occurs with *Ophioglossum vulgatum* in all five sites, *Acer rubrum* and *Onoclea sensibilis* are abundant in four of the sites and *Achillea millefolium*, *Spiraea latifolia* and *Thelypteris palustris*, are abundant in three of the sites. Sporophytes of *O. vulgatum* often are found growing in the shade of small shrubs, usually *Alnus* spp., *Cornus* spp., *Salix* spp., or *Spiraea* spp. In the Brewster site the single *O. vulgatum* sporophyte occurred beneath *Leucothoë racemosa*.

Observation of populations over several growing seasons suggest that where the shrub layer is completely lacking, aggressive grass, sedge and rush species predominate and *Ophioglossum vulgatum* declines. The shrub layer, when present, may help to control competition from those species. *Ophioglossum vulgatum* seems to prefer sites where the shrub layer is present but constrained by pruning or periodic flooding.

Populations of *Ophioglossum vulgatum* are capable of sudden and dramatic increases or declines. The current Conway site appears to have increased from 26 sporophytes in 1980 to a maximum of 901 in 1991, the Lenox site from 0 to 205 in under 12 years, and the Boylston site from 30 or 40 to 109 in three years. Conversely, the Rowe population of approximately 150 sporophytes disappeared completely after the 1988 growing season and the Brewster population of 19 in 1985 had declined to only one in 1992. Five additional more gradual extinctions have been documented in the state since 1961.

The low levels of genetic variability revealed by electrophoresis may be important factors in the decline of *Ophioglossum vulgatum* in Massachusetts. A combination of intragametophytic selfing, small population size, low numbers of reproductively mature sporophytes, founder effect and specialized habitat requirements may be responsible for the loss of genetic variability within and among populations. These processes may in turn lead to an inability to adapt to environmental change in an inherently volatile habitat. While *O. vulgatum* seems to be well adapted to compete in early successional sites in Massachusetts, it may lack the genetic heterogeneity that would allow it to persist as these sites change. Only at the Conway site where succession is inhibited by intermittent flooding does *O. vulgatum* appear to thrive. Hence its long-term survival in Massachusetts may be a function not of adaptation to changing conditions in a site, but of its ability to disperse to new, sometimes distant sites and to found new populations successfully.

The habitat requirements and reproductive biology of *Ophioglossum vulgatum* described suggest a number of measures for the management of existing populations.

(1) Plant species competing with *Ophioglossum vulgatum* should be controlled. Shrubs and small trees in the immediate vicinity should be selectively pruned to provide moderate levels of filtered light. The surrounding forest canopy may also require periodic

thinning. Because the effects of herbicides on *O. vulgatum* are not known, their use to suppress surrounding vegetation is not recommended. However, most populations of *O. vulgatum* are small enough that competing vegetation can be controlled by mechanical means such as a portable "weed-wacker" or even hand clippers. The invasion of grasses, sedges and rushes may be restricted by mowing late in the growing season, probably after October 1. Care should be taken to protect shrubs and small trees from damage during mowing.

(2) Disruption or compaction of soil should be avoided. Heavy mowing equipment should not be used to clear *Ophioglossum vulgatum* sites. Footpaths and vehicle rights-of-way should be diverted to provide a generous buffer zone around protected populations.

(3) Proper hydrological conditions in the immediate vicinity of the *Ophioglossum vulgatum* population should be maintained. Extended inundation or desiccation of the substrate during the growing season which may result from beaver activity or anthropogenic activities such as draining and filling often associated with agriculture and highway construction should be avoided.

The size of an *Ophioglossum vulgatum* population may fluctuate dramatically from year to year depending on availability of light, soil moisture, disturbance and other conditions. Because of the extensive root network and the ability of a population to expand vegetatively, it may also be important to protect and manage a site even after all sporophytes have disappeared. It is possible that a subsequent reversal of environmental conditions could result in the development of new sporophytes from the dormant root system. Germination of spores or gametophytes may also occur after an extended period of dormancy.

Habitat for *Ophioglossum vulgatum* also may be provided in sites where it does not occur at present, particularly when those sites are near existing populations. Minimum requisite conditions should include a saturated substrate and moderate to high light levels. Sites where such conditions occur naturally may include seeps, wet meadows and abandoned beaver meadows. Areas subject to some human disturbance also may be appropriate including highway shoulders or median strips, golf courses, utility corridors, or pastures.

While historical data suggest that *Ophioglossum vulgatum* has never been abundant in Massachusetts, its present status is pre-

carious. Of the five known populations, the Brewster population with its single sporophyte is probably destined for extinction, while the Boylston, Lenox and Sunderland populations are marginal. The Conway population, while relatively large and healthy in 1991 and 1992, could easily be subject to the kind of sudden decline that has been observed at other sites.

Continued availability of disturbed, early successional sites may be important to the survival of *Ophioglossum vulgatum* in Massachusetts. These sites may result from naturally occurring disturbances such as wildfires, windstorms, debris slides and insect predation. Some anthropogenic factors such as highway and dam construction may favor the creation of disturbed sites while others, such as the decline of agriculture and suppression of wildfires, may result in less disturbance. While the reintroduction of beavers into the state in the 1930's created new early successional sites, the subsequent expansion of beaver populations may have had the opposite effect, forcing beavers to reoccupy abandoned sites before early successional species were able to colonize them. Thus human activity can be either beneficial or harmful to the survival of *O. vulgatum* in Massachusetts.

Efforts should be made to locate additional populations of *Ophioglossum vulgatum* in Massachusetts. Hagenah (1966) writes "[*Ophioglossum vulgatum*] blends with the grasses and other vegetation, but after the first one is noticed search may reveal scores or even hundreds more." Similarly, Wagner (1971) observes that "Many botanists and naturalists regard the adder's-tongue as a rare plant, but this is probably due largely to the fact that it is overlooked." Roberta Poland (MASS) located at least 11 stations for *O. vulgatum* in the town of Deerfield alone during the 1950's, an achievement which should inspire continued persistence in the search for this ancient and intriguing plant.

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DEPARTMENT OF BIOLOGICAL SCIENCES
SMITH COLLEGE
NORTHAMPTON, MA 01060